Takara Bio USA

Lenti-X™ p24 Rapid Titer Kit (Single Wash) User Manual

Cat. No. 631476

(050323)

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I. Introduction

Principle of the Lenti-X p24 Rapid Titer Kit (Single Wash)

Lenti-X p24 Rapid Titer Kit (Single Wash) (Cat. No. 631476) provides a quick assay for determining the titer of any HIV-1-based lentiviral supernatant. The assay utilizes an Enzyme Linked Immunosorbent Assay (ELISA) to detect the presence of p24 in a lentiviral sample. The p24 protein is encoded by the HIV-1 *gag* gene, which is the major lentiviral capsid protein (Figure 1). This product comes with a 96 well plate that is coated with a p24 specific capture antibody. To use the kit, a mixture of the lentiviral sample and a second horseradish peroxidase (HRP) conjugated-anti-p24 detection antibody is added to the wells. The p24 protein in the sample binds to the capture antibody on the plate and is labeled by the HRP-anti-p24 detection antibody (Figure 2). A single washing procedure is used to remove any unbound anti-p24 detection antibody. After adding substrate, the color intensity is measured spectrophotometrically to determine the level of p24 in the samples, which can be precisely quantified against a p24 standard curve. p24 values can then be correlated to the virus titer of packaging cell supernatants.

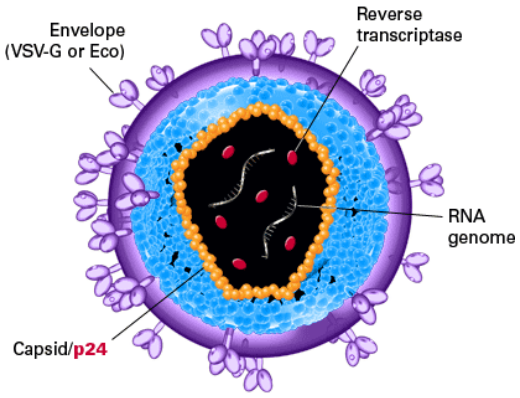

Figure 1. Lentiviral p24 is a virus core/capsid protein. The HIV-1 *gag* gene encodes p24, which is the major lentiviral capsid protein. The lysis of viral particles is necessary to generate soluble p24 which can then be measured by this kit.

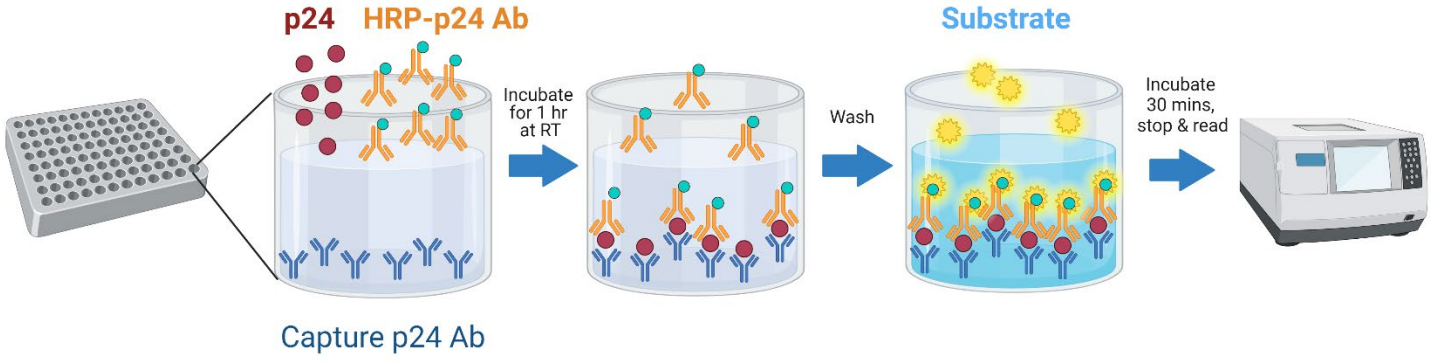

Figure 2. The Lenti-X p24 Rapid Titer Kit (Single Wash) workflow. Lentiviral p24 core protein in packaging cell supernatants is bound to wells of a microtiter plate coated with p24 capture antibody. The presence of bound p24 in the wells is detected using a HRP conjugated secondary anti-p24 antibody, and a color-producing substrate. Quantitation is performed by comparing test samples to a p24 standard curve.

II. List of Components

Lenti-X p24 Rapid Titer Kit (Single Wash) (Cat. No. 631476) contains sufficient reagents to perform 96 reactions.

NOTE: Do not use reagents beyond the expiration date on the kit label.

Table 1. Lenti-X p24 Rapid Titer Kit (Single Wash) components.

Lenti-X p24 Rapid Titer Kit (Single Wash) (Store at 4°C)	631476
Anti-p24 Coated Plate (12 x 8-well microtiter strips)*	1 plate
p24 Control	500 ng
p24 Reconstitution Buffer	1.5 ml
Diluent Buffer	20 ml
Lysis Buffer	4 ml
Anti-p24 (HRP Conjugate)	12 ml
Washing Buffer (20X)†	50 ml
TMB Substrate	12 ml
Stop Solution	12 ml

* Once opened, microtiter strips may be stored at 2–8°C until the expiration date on the label. Microtiter strips must be stored under desiccated conditions. Return unused microtiter strips to their original foil pouch along with the desiccant sachet, and securely reseal the pouch by folding over the open end and securing it with adhesive tape.

†The 20X Wash Buffer can develop a crystalline precipitate during initial storage at 4°C. This will dissolve upon warming to 37°C. After first use, store 20X Wash Buffer at room temperature.

III. Additional Materials Required (Not Provided)

The following materials, or their equivalents, are required to perform the Lenti-X p24 Rapid Titer Kit (Single Wash) assay:

- Micropipettes for delivering volumes of 2 µl, 20 µl, 100 µl, 200 µl, and 1,000 µl. A multichannel pipette is preferred for dispensing reagents into microtiter plates.
- Filtered pipette tips for 1 ml or larger pipettes
- Distilled or deionized water
- 15 ml and 100 ml beakers
- 1 L graduated cylinder
- 1 ml, 5 ml, 10 ml, and 25 ml pipettes
- 25 ml reagent reservoirs
- Microtube rack
- 2 ml centrifuge tubes
- Automatic microtiter plate washer or a vacuum line fitted with a vacuum pump trap to collect liquid
- Absorbent paper towels
- Microtiter plate reader with 450 nm filter
- Latex gloves, safety glasses, and other appropriate protective garments
- Biohazard infectious waste containers
- Timer

IV. General Considerations and Safety Guidelines

A. General Requirements

- The Lenti-X p24 Rapid Titer Kit (Single Wash) contains reagents which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
- Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of the microtiter strips intended for future use.
- Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about midsection. Follow manufacturer's recommendations for automatic processors.
- Always keep the upper surface of the microtiter strips free from excess fluid droplets. Reagents and buffer overspill should be blotted dry on completion of the manipulation.
- Do not allow the wells to completely dry during an assay.

B. Sample Recommendations and Requirements

- The Lenti-X p24 Rapid Titer Kit (Single Wash) is intended for use with tissue culture supernatants. The performance characteristics have not been established for any matrices other than tissue culture media. See the Appendix for more information.
- Specimens should be tested as soon as possible but can also be stored frozen at –80°C, if necessary.
- Thoroughly mix thawed samples before testing.
- The assay cannot be used to quantitate samples having p24 values greater than the highest value on the p24 standard curve, unless the samples are diluted sufficiently. See the Appendix for more information.

C. Lentivirus Advisory

For your safety and the safety of others around you, it is imperative to fully understand the potential hazards of working with recombinant lentiviruses and the necessary precautions for their use in the laboratory.

The National Institute of Health and Center for Disease Control have designated recombinant lentiviruses as Level 2 organisms. This requires the maintenance of a Biosafety Level 2 facility for work involving this virus and others like it. The VSV-G pseudotyped lentiviruses packaged from the HIV-1-based vectors described here are capable of infecting human cells. The viral supernatants produced by these lentiviral systems could, depending on your insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes in vivo. **For these reasons, due caution must be exercised in the production and handling of any recombinant lentivirus. The user is strongly advised not to create VSV-G pseudotyped lentiviruses capable of expressing known oncogenes.**

For more information on Biosafety Level 2 agents and practices, download the following reference:

CDC & NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC. *U.S. Dep. Heal. Hum. Serv.* (2020). at https://www.cdc.gov/labs/BMBL.html

D. Biosafety Level 2

The following is a brief description of Biosafety Level 2. It is neither detailed nor complete. Details of the practices, safety equipment, and facilities that combine to produce Biosafety Level 2 are available in the publication link above. If possible, observe and learn the practices described below from someone who has experience working with lentiviruses.

Important Features of Biosafety Level 2:

Practices:

- Standard microbiological practices
- Limit access to work area
- Post biohazard warning signs
- Minimize production of aerosols
- Decontaminate potentially infectious wastes before disposal
- Use precautions with sharps (e.g., syringes, blades)
- Access to biosafety manual defining any needed waste decontamination or medical surveillance policies

Safety equipment:

- Biological safety cabinet, preferably a Class II BSC/laminar flow hood (with a HEPA microfilter) used for all manipulations of agents that cause splashes or aerosols of infectious materials; exhaust air is not recirculated
- PPE: protective laboratory coats, gloves, face protection as needed

Facilities:

- Autoclave for waste decontamination
- Chemical disinfectants for spills

V. Protocol: Lenti-X p24 Rapid Titer Kit (Single Wash) Procedures

IMPORTANT: Please read the entire protocol before starting. Detailed instructions are provided for the quantitative assay of p24.

A. Protocol: Wash Buffer Preparation

NOTE: The 20X Wash Buffer can develop a crystalline precipitate during storage at 2–8°C. This will dissolve upon warming to 37°C. After first use, store 20X Wash Buffer at room temperature.

Prepare 1X wash buffer by diluting 1 part Wash Buffer (20X) with 19 parts distilled or deionized water.

- We recommend that fresh wash buffer be prepared before each assay, but the working strength (1X) of wash buffer can be stored up to 3 weeks at 2–8°C, if necessary. If the kit will be utilized over a period greater than four weeks, then prepare only enough working strength wash buffer for what is immediately needed.
- If the diluted buffer becomes visibly cloudy or develops a precipitate during storage, discard it and prepare fresh buffer.
- If manual plate washing is used, each microtiter strip of 8-wells can be adequately washed with ~20 ml of working strength wash buffer.

B. Protocol: Preparing p24 Standard Curve and Sample Dilutions

1. Protocol: Preparing Dilutions for p24 Standard Curve

To test samples quantitatively and determine accurate virus titers, you will need to prepare a p24 standard curve (0–1,600 pg/ml) and a serial dilution of the sample(s). Examples of data collected for a typical standard curve and for test samples are shown in Table 2 and Figure 5.

1. Allow all reagents to reach room temperature (18–25°C).

NOTE: Refer to Figure 3 for a visual aid of Steps 2–4.

2. Prepare p24 stock solution by adding 1.25 ml of Reconstitution Buffer to p24 Control (500 ng, lyophilized powder), and vortex for 5 sec to make a 400 ng/ml stock solution.

   NOTE: The reconstituted p24 stock (400 ng/ml) is stable for 4 weeks when stored at 4˚C.

3. Label a 2 ml centrifuge tube as 1,600 pg/ml and dispense 996 µl of Diluent Buffer into it.
4. Add 4 µl the p24 control stock to the labeled 2 ml tube from Step 3, and mix (Total: 1 ml).

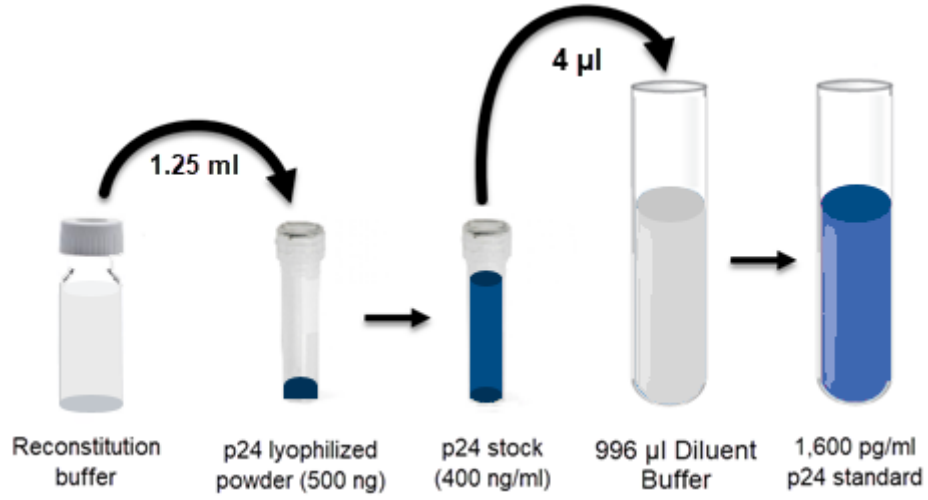

Figure 3. Preparing the p24 stock solution and 1,600 pg/ml p24 standard solution.

5. Prepare five more centrifuge tubes by labeling them for the following concentrations: 800 pg/ml, 400 pg/ml, 200 pg/ml, 100 pg/ml, and negative control (NTC; 0 pg/ml).
6. Dispense 500 µl of Diluent Buffer into each of the tubes labeled in Step 5. Set aside the NTC tube for now; this will be used again when performing the ELISA (Section V.C).

NOTE: Refer to Figure 4 for a visual aid of Steps 7–10.

7. Add 500 µl of the 1,600 pg/ml solution (prepared in Step 4) into the 800 pg/ml tube and mix.
8. Using a fresh pipette tip, transfer 500 µl of the 800 pg/ml solution into the 400 pg/ml tube and mix.
9. Using a fresh tip, transfer 500 µl of the 400 pg/ml solution into the 200 pg/ml tube and mix.
10. Using a fresh tip, transfer 500 µl of the 200 pg/ml solution into the 100 pg/ml tube and mix.

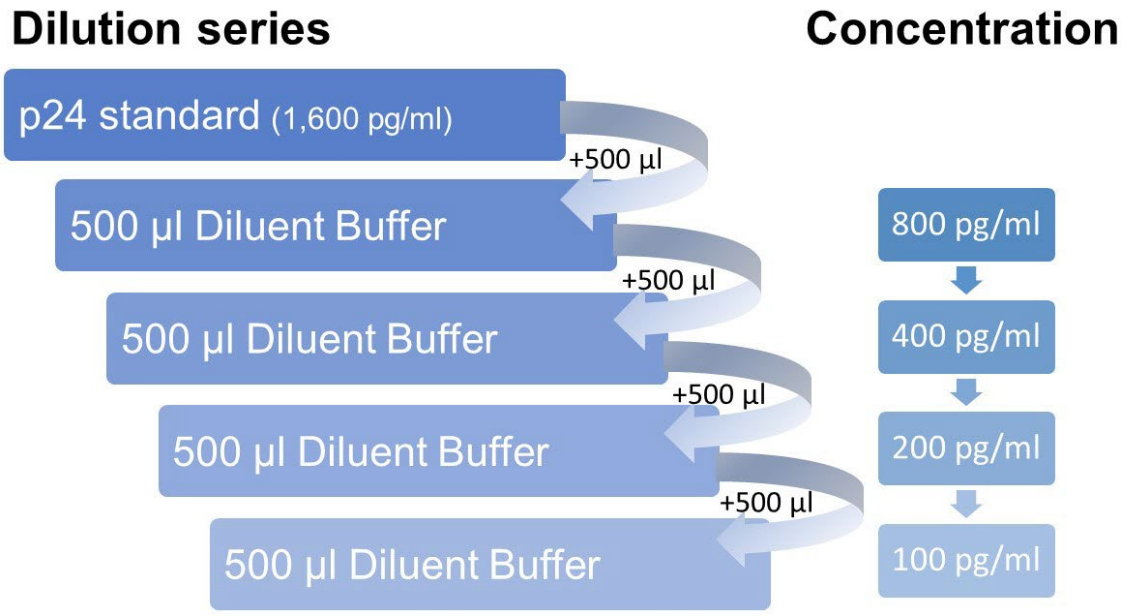

Figure 4. Preparing the p24 curve serial dilutions. 500 µl of the previous concentration solution is added to 500 µl of Diluent Buffer in a fresh tube to make each subsequent solution for the standard curve.

2. Protocol: Preparing Dilutions of the Sample(s)

To obtain accurate p24 values, samples containing high levels of p24 (i.e., >1,600 pg/ml) must be diluted prior to assay. Such samples may include lentiviral supernatants produced using Lenti-X Packaging Single Shots (Cat. Nos. 631275–631278, 631282, or 631294), which often require diluting 10–10,000-fold.

We recommend making several serial, tenfold dilutions to generate at least one dilution in the range of the standard curve. Mix dilutions thoroughly before assaying or diluting them further, assay each sample in duplicate, and be sure to multiply each result by its dilution factor to determine the correct p24 value in the original sample.

C. Protocol: Perform ELISA (~1.5 hr)

1. Select enough 8-well microtiter strips to accommodate all standards, test specimens, controls, and Diluent Buffer (NTCs) in duplicate.
2. Fit the microtiter strips into a holding frame.
3. Assign wells based to sample identity using the molded letter/number cross reference system on the plastic frame.
4. Add 100 µl of the p24 standards or sample dilutions (prepared in Section V.B) to its respective labeled 2 ml centrifuge tube.

   NOTE: For consistency, we recommend preparing the sample mixture in a tube before transferring to the ELISA plate.

5. Add 20 µl of Lysis Buffer to each tube then mix.
6. Add 100 µl of the Anti-p24 (HRP conjugate) to each tube then mix.
7. Dispense 200 µl of each test sample into the designated wells assigned for the p24 standard and sample.
8. Incubate the used microtiter strips at room temperature (18–25°C) for 60 min ± 5 min.

9. Aspirate the contents of the wells and wash the microtiter plate either via automatic or manual plate washing, following the appropriate protocol below.

NOTES:
- Using an automatic plate washer is recommended to enhance speed, efficiency, and well-to-well consistency. Manual plate washing can yield equivalent results if performed carefully.
- Each Lenti-X p24 assay requires performing a single six-rinse wash cycle.

a. **Manual Plate Washing**

For manual plate washing, perform the following steps for the rinse cycle:

i. Use a vacuum line fitted with a vacuum pump trap to aspirate liquid from the sides of all wells.
ii. With a multichannel pipette or a laboratory squeeze bottle, fill wells with ~300 µl of Washing Buffer (to the brim).
iii. Aspirate the wells with the vacuum line.
iv. Repeat steps ii. and iii. five more times (Total: 6 rinse/wash cycles)
v. Flip the plate well-side down and tap firmly on a paper towel to remove any excess liquid.

b. **Automatic Plate Washing**

i. The rinse cycle must consist of six consecutive washes.
ii. After the final rinse cycle, flip the plate side down and tap firmly.
iii. Check for any residual wash buffer in the wells and blot the upper surface with a dry paper towel.

Automatic plate washers should meet the following criteria:

- All wells must be completely aspirated.
- All wells are filled with ~300 µl Washing Buffer during each rinse cycle.
- The apparatus must be regularly cleaned following the manufacturer's instructions to prevent contamination.

10. After washing, without delay, use a multichannel pipette to dispense 100 µl of TMB Substrate Solution into each well.
11. Protect the plate from direct light/sunlight and incubate at room temperature (18–25°C) for 30 ± 2 min.
12. Stop the reaction by adding 100 µl of Stop Solution to each well.
13. Mix on a plate shaker for 5–10 sec or tap lightly. The blue solution should change to a homogeneous yellow color in each well.
14. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
15. Immediately read the absorbance values at 450 nm using a microtiter plate reader.

Table 2. Sample data for standard curve

Standard (pg/ml)	Absorbance (450 nm)			Mean	Sample (pg/ml)
	Rep 1	Rep 2	Rep 3		
0	0.0412	0.0405	0.0411	0.0409	–
100	0.1460	0.1330	0.1385	0.1392	–
200	0.2811	0.3185	0.3254	0.3084	–
400	0.5970	0.5957	0.6286	0.6071	–
800	1.1908	1.2211	1.2175	1.2098	–
1600	2.2749	2.2765	2.3836	2.3117	–
Sample 1	0.3211	0.3013	0.2846	0.3023	196.8
Sample 2	0.1875	0.1617	0.1669	0.1720	103.7
Sample 3	1.5799	1.6416	1.5020	1.5745	1,105.5

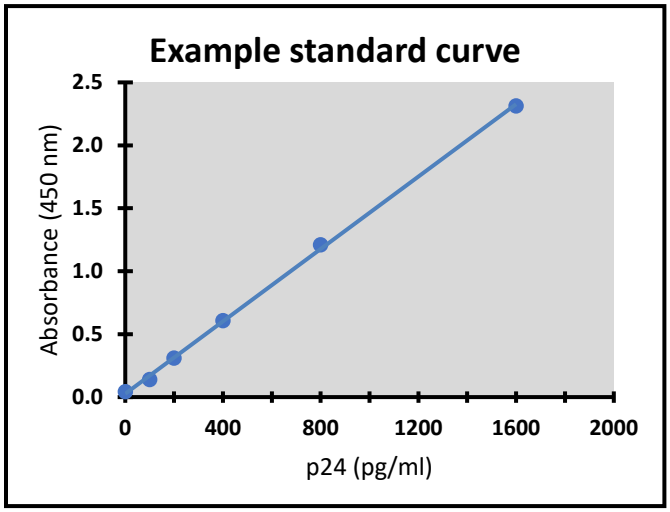

Figure 5. Lenti-X p24 Rapid Titer Kit (Single Wash) standard curve. The curve was generated using the p24 standards and results listed in Table 2. Samples were prepared and assayed as described.

VI. Determining Your Virus Titer

Your p24 values can be used to determine the relative virus titers of your packaging cell supernatants. To calibrate your virus production system and determine a relationship between p24 levels and infectivity, it may be useful to determine the p24 levels of supernatants for which you have already measured the virus titer using an alternative method (i.e., determining infectious units based on expression of a fluorescent protein or drug-selective marker).

Example: the following values and calculations may also be used to determine approximate titers and are based on the observation that each lentiviral particle (LP) contains approximately 2,000 molecules of p24:

- 1 LP contains 8×10^{-5} pg of p24, derived from:

$$\frac{2{,}000 \times (24 \times 10^{3}\ \text{Da})}{6 \times 10^{23}}$$

- 1 ng p24 is equivalent to ~1.25×10^{7} LPs
- For a typical lentivirus vector, there is 1 IFU for every 100–1,000 LPs
- Therefore, a supernatant titer of 10^{7} IFU/ml ≈ 10^{9}–10^{10} LP/ml or 80–800 ng p24/ml

Appendix. Troubleshooting Guide

Table 3. Troubleshooting guide

Problem	Possible Explanation	Solution
Reagents become visibly cloudy or contain a precipitate.	The 20X Wash Buffer can develop a crystalline precipitate during storage at 2–8°C.	Precipitate will dissolve upon warming the 20X Wash Buffer to 37°C.
	For other reagents, this may be caused by a kit with previously opened reagents or past its expiration date.	Use a new kit with fresh reagents. If this is a newly opened kit not past the expiration date, contact technical support.
The TMB Substrate solution becomes dark blue in color.	This is likely caused by chemical contamination of the substrate solution.	Use a new kit with fresh reagents. If this is a newly opened kit not past the expiration date, contact technical support.
The assay cannot be used to quantitate samples having p24 values greater than the highest value on the p24 standard curve	The samples are not diluted sufficiently	Perform serial dilution of the sample until the dilution falls into the p24 standard curve.
A non-tissue culture media was used as a sample source.	The performance characteristics have not been established for any matrices other than tissue culture media	Use only tissue culture media as your sample.
The kit fails to meet the required criteria for a valid test	Check possible issues in the table above.	If no issues match your experimental situation, contact technical support.

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